Showcasing research into mammalian metabolism of organic selenium compounds by Professor Naoki Furuta in the Department of Applied Chemistry at Chuo University, Tokyo, Japan and colleagues in the Department of Epidemiology and Environmental Health, Department of Internal Medicine, Juntendo University School of Medicine, Tokyo, Japan.

Selenium metabolism and excretion in mice after injection of $^{82}$Se-enriched selenomethionine

Organic selenium compounds in plants and yeasts are effective chemoprotectants in mammalian cancer. We identified selenomethionine pathways by measuring endogenous and exogenous $^{82}$Se levels and quantified selenium compounds and selenoproteins in mice liver, kidneys, plasma and urine.

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Selenium metabolism and excretion in mice after injection of $^{82}$Se-enriched selenomethionine†

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The organic Se compounds (particularly selenomethionine [SeMet]) in plants and yeasts are very effective chemoprotectants for mammalian cancer. To characterize the dynamics of selenomethionine utilization pathways, we intravenously injected $^{82}$Se-enriched SeMet into mice under different nutritional states (Se-adequate and Se-deficient mice) and then measured their endogenous and exogenous $^{82}$Se levels. Furthermore, we quantified Se compounds and selenoproteins in liver, kidneys, plasma, and urine. The average recoveries of exogenous $^{82}$Se from solid tissues, urine, and feces were 81% for Se-adequate mice and 84% for Se-deficient mice. Exogenous $^{82}$Se was distributed in the hepatic and renal cytosols as cellular glutathione peroxidase (cGPx), selenosugar, and SeMet within 1 h after injection. Synthesis of cGPx was maintained until 72 h after injection, regardless of the Se nutritional status. Whereas plasma levels of exogenous $^{82}$Se as selenoprotein P (Sel-P) peaked at 6 h after injection, those of Se-containing albumin (SeAlb), extracellular GPx, and SeMet peaked at 1 h after injection. These results suggest three Se transport pathways in mice injected with SeMet: SeAlb (within 1 h after injection of $^{82}$Se-enriched selenomethionine [SeMet]); SeMet (from 1 to 72 h after injection); and Sel-P (from 6 to 72 h after injection). The amount of Sel-P in Se-deficient mice was 1.5 times that of Se-adequate mice, and this increase was much larger than Se-containing compounds other than Sel-P. Our results indicate that Sel-P has an important role in Se transport when the nutritional supply of Se is insufficient.

Introduction

Selenium (Se) is an essential element for humans and other animals and plays important roles in the (i) generation of an antioxidant effect, (ii) alleviation of heavy-metal toxicity, and (iii) production of sperm. The levels of Se necessary for and toxic to human life are quite close to each other. Se deficiency induces arterial sclerosis and cardiac myopathy, and exacerbates infectious disease. Epidemiologic data suggest that dietary Se deficiency is associated with an increased risk of cancer. The largest-ever prostate cancer-prevention clinical trial (SELECT: the Selenium and Vitamin E Cancer Prevention Trial) began enrolling patients on August 22, 2001; enrollment closed on June 24, 2004, after 35,534 participants had been enrolled. Initial data from SELECT, reported in late 2008, showed that daily selenium and vitamin E supplements, taken either alone or together for a median of 5.5 years, did not prevent prostate cancer. However, additional data from SELECT showed that the absolute increase in risk of prostate cancer per 1000 person-years was 1.6 after supplementation with vitamin E, 0.8 after selenium supplementation, and 0.4 for the combination, compared with placebo. Nevertheless, numerous studies have reported that the organic Se compounds in plants and yeasts are very effective in the chemoprevention of mammalian cancer. Therefore, the use of these organic Se compounds as medicinal chemicals merits exploration.

To characterize Se metabolism, tracer experiments with enriched stable Se isotopes and Se speciation analysis using high-performance liquid chromatography (HPLC) coupled with inductively-coupled plasma mass spectrometry (ICPMS) have been performed. Enriched stable Se isotopes were administered to rats or mice in the chemical forms of selenite (Se(v)), selenate (Se(vi)), selenomethionine (SeMet), methylselenocysteine, selenosugar, methylseleninic acid, and dimethylselenoxide. Most of these works focused on time-dependent changes of these Se compounds and provided only

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qualitative or semi-quantitative data. Moreover, the regulation of Se metabolic pathways under various Se nutritional states had not been investigated previously.

In the current study, we separately quantified the amounts of endogenous and exogenous \(^{82}\)Se in 13 solid tissues, 3 body fluids (red blood cells [RBCs], plasma, and urine), and the feces of mice after the injection of \(^{82}\)Se-enriched \(^{82}\)SeMet intravenously. Furthermore, we quantified the amounts of various Se species, including cellular glutathione peroxidase (cGPx), selenoprotein P (SeP-P [the official gene symbol for selenoprotein P is Sepp/SePP]), selenosugar, and SeMet, and then evaluated the Se dynamic pathways under different selenium nutritional states. Finally, we compared the metabolic pathways of SeMet with data from our previous experiments of \(^{82}\)Se uptake in mice.\(^{22,24}\)

**Experimental**

**Chemical synthesis**

\(^{82}\)Se metal powder (\(^{75}\)Se 0.00%, \(^{76}\)Se 0.08%, \(^{77}\)Se 0.02%, \(^{78}\)Se 0.03%, \(^{80}\)Se 0.15%, \(^{82}\)Se 99.72%) was purchased from Eurisotope, Gif-sur-Yvette, France. As done previously,\(^{27}\) \(^{82}\)Se-enriched \(^{15}\)SeMet was synthesized from 6 mL of 1.2 mol L\(^{-1}\) methyllithium in diethyl ether solution, 30 mL of \(^{82}\)Se (100 mg, 1.2 mmol) in dry tetrahydrofuran, and 10 mL of (S)-2-amino-4-bromobutanoic acid hydrobromide (400 mg, 1.5 mmol) in 100% ethanol. After evaporation of the solvent under reduced pressure, the residue was dissolved in 1 mol L\(^{-1}\) HCl (50 mL) and washed with diethyl ether (twice, 30 mL each wash). The aqueous layer was neutralized with 1 mol L\(^{-1}\) NaOH. The solution was applied to a cation-exchange Dowex 50W X8 column (inner diameter, 20 mm; length, 130 mm; H\(^{+}\) form; Wako Pure Chemical Industries, Ltd., Osaka, Japan), washed with water (200 mL), and eluted with 1 mol L\(^{-1}\) ammonia (200 mL). After evaporation of the solvent under reduced pressure, the crude product was obtained as a colorless solid, which was recrystallized using water–ethanol to yield \(^{82}\)Se enriched \(^{15}\)SeMet (80.4 mg, 0.40 mmol, 33.4% from \(^{82}\)Se), a white crystalline solid.

**Animal experiments**

Animal experiments were performed under approval from the Animal Experiment Ethical Committee, Juntendo University School of Medicine (approval number, 210068). Male mice (Crlj::CD1(ICR); age, 4 weeks; n = 30) were purchased from Charles River Laboratories Japan (Kanagawa, Japan). The mice were fed with a Se-adequate diet (Se-adequate mice); the other group of mice (15 mice) received a Se-deficient diet (Se-deficient mice) for 1 week before beginning the dietary modification experiment. One group of mice (15 mice) continued to be fed with the Se-adequate diet (Se-adequate mice); the other group (15 mice) received a Se-deficient diet (<0.05 \(\mu\)g Se g\(^{-1}\); <0.25 \(\mu\)g Se per day) for the following 4 weeks (Se-deficient mice). After 4 weeks, each mouse was injected intravenously with 0.2 mL of \(^{82}\)Se-enriched \(^{82}\)SeMet solution (containing 11.25 \(\mu\)g \(^{82}\)Se ml\(^{-1}\) as \(^{82}\)SeMet). The mice continued to receive the Se-adequate and Se-deficient diets, respectively, for 72 h after injection.

Three mice from each group were euthanized with diethyl ether and dissected just before injection (0 h) and at 1, 6, 24, and 72 h after injection, and the liver, kidneys, muscle, spleen, pancreas, heart, lungs, thymus, submandibular glands, brain, testes, epididymides, seminal vesicles and red blood cells (RBCs), plasma were collected. Livers were perfused with 5% glucose (special reagent grade, Wako Pure Chemical Industries) prior to further analysis. Samples of urine and feces were collected using a metabolic cage (type MC, Sugiyama-Gen Iriki Company, Tokyo, Japan).

**Sample preparation**

Portions of liver and kidney (about 0.5 and 0.3 g, respectively) were homogenized in 2 volumes of 50 mmol L\(^{-1}\) Tris-H\(_2\)NO\(_3\) buffer solution (pH 7.4) using a glass–Teflon homogenizer (model HOM, As One Company, Osaka, Japan) while being chilled in an ice bath. The homogenate then was ultracentrifuged at 105,000 \(\times\) g for 60 min at 4 °C, and the cytosolic fraction was recovered and used for size-exclusion chromatography.

Samples (1 mL each) of cytosolic fraction were diluted with 1 mL of a mixed ion-pair reagent (2.5 mmol L\(^{-1}\) sodium 1-butane-sulfonate and 8 mmol L\(^{-1}\) tetramethylammonium hydroxide, 4 mmol L\(^{-1}\) malonic acid, and 0.05% methanol [pH 4.5]). Denatured protein was removed by centrifuging at 4000 \(\times\) g for 10 min. All samples were filtered through nitrocellulose membrane filters (pore diameter, 0.45 \(\mu\)m; Nihon Millipore, Tokyo, Japan) prior to Se speciation analysis by HPLC–ICPMS.

**Determination of total Se concentration in solid tissues, body fluids, and feces**

As done previously,\(^{22}\) samples (approximately 0.1 g each) of tissues, RBCs, plasma, urine, and feces of mice each were mixed with 400 \(\mu\)L of HNO\(_3\) and 200 \(\mu\)L of H\(_2\)O\(_2\) and digested using a microwave oven (MLS-1200 MEGA; rotor, MDR-300/s; Milestone General, Tokyo, Japan). After samples were completely digested, indium was added to a final concentration of 50 ng mL\(^{-1}\) for use as an internal standard, and the weight was adjusted to 1.5 g using ultrapure water. Se content was determined after correcting for spectral interference due to \(^{40}\)Ar\(^{35}\)Cl and \(^{81}\)Br\(^{1}H\) and for signal enhancement due to co-existing carbon and after internal standardization as described previously.\(^{22}\) Total muscle weight was calculated by multiplying the body weight by 0.42.\(^{29}\)

**Se speciation analysis using HPLC–ICPMS**

An HPLC system consisting of a degasser (DG-158053, Jasco, Tokyo, Japan), a pump (PU-1580i, Jasco), and a sample injector (model 9725i, Rheodyne, Rhonert Park, CA, USA) was used for speciation analysis of hepatic and renal cytosolic fractions. For speciation analysis of plasma, we used a capillary liquid chromatographic system consisting of a degasser (DG661, GL Sciences, Tokyo, Japan), an HPLC system (9725i, Rheodyne, Rhonert Park, CA, USA) was used for speciation analysis of plasma, we used a capillary liquid chromatographic system consisting of a degasser (DG661, GL Sciences, Tokyo, Japan), an HPLC–ICPMS system (HP 4500, Agilent Technologies, Tokyo, Japan) was used to measure \(^{77}\)Se and \(^{82}\)Se.

A size-exclusion column (Asahipak GS-520 7G; inner diameter, 7.5 mm; length, 500 mm; Showa Denko, Tokyo, Japan) with a
guard column (Asahipak GS-2G 7B; inner diameter, 7.5 mm; length, 50 mm; Showa Denko) was used to separate the Se compounds in hepatic and renal cytosolic fractions. Aliquots (100 μL each) of hepatic and renal cytosolic fractions were injected onto this column, and 50 mmol L⁻¹ Tris-HClO₄ buffer solution (pH 7.4) was used for elution at a flow rate of 1.0 mL min⁻¹.

A reverse-phase column (Lichrosorb RP-18; inner diameter, 4.6 mm; length, 250 mm; Seibersdorf, Austria) was used for the separation of SeMet from selenosugar. A mixed ion-pair reagent (2.5 mmol L⁻¹ sodium 1-butanesulfonate and 8 mmol L⁻¹ tetramethylammonium hydroxide, 4 mmol L⁻¹ malonic acid, and 0.05% methanol [pH 4.5]) was used as the mobile phase.³⁰ Aliquots (100 μL each) of hepatic and renal cytosolic fractions, which were diluted with the mobile phase and centrifuged, were injected onto this column. For size-exclusion and reverse phase columns, the eluate was introduced directly into an ICPMS. A concentric nebulizer (AR35-1-FC1, Glass Expansion, West Melbourne, VIC, Australia) was used to introduce the eluate into ICP from the columns.

A microaffinity column (Afpak AH-R-94; inner diameter, 0.5 mm; length, 35 mm; Showa Denko), packed with heparin, was used for speciation of Se in plasma. This column has affinity for histidine residues; among Se-containing proteins in plasma, only Sel-P is selectively retained on heparin.³¹,³² To elute the adsorbed Sel-P, the mobile phase was switched at 180 s from equilibration buffer (20 mmol L⁻¹ Tris-HClO₄; pH 7.4) to elution buffer (20 mmol L⁻¹ Tris-HNO₃ + 1.4 mol L⁻¹ ammonium acetate; pH 7.4) using a step gradient.

To achieve the necessary low flow rate (6 μL min⁻¹) for speciation analysis of plasma, a total consumption nebulizer (CEI-100, CETAC, Omaha, NE, USA) was used. For connecting between an injector and the microaffinity column, and between the column and the total consumption nebulizer, fused-silica tubes (inner diameter, 50 μm; outer diameter, 375 μm; GL Sciences) were used.

The concentrations of Se compounds were determined by injecting one-point analytical standards of cGPx (Sigma-Aldrich, Saint Quentin Fallavier, France), selenite (Nacalai Tesque, Kyoto, Japan), selenomethionine (Sigma-Aldrich), and 1β-methylseleno-N-acetyl-β-D-galactosamine (selenosugar) (which was kindly provided by Prof. Kazuo T. Suzuki and Prof. Yasumitsu Ogra), respectively, after signals were integrated using ICPMS ChemStation (B.03.05, Agilent Technologies).

The concentrations of endogenous and exogenous Se were calculated from m/z counts of ⁷⁷Se and ⁸²Se, as described previously.²² The relationship between the concentrations of total exogenous Se and exogenous ⁸²Se and between those of total endogenous Se and endogenous ⁸²Se is expressed as follows:

\[
\text{Exogenous } ⁸²\text{Se} = \text{exogenous Se} \times ⁸²\text{Se isotopic abundance (0.9972)}
\]

\[
\text{Endogenous } ⁸²\text{Se} = \text{endogenous Se} \times ⁸²\text{Se isotopic abundance (0.0873)}
\]

Therefore, exogenous Se is almost the same as exogenous ⁸²Se. It can be calculated by multiplying exogenous ⁸²Se by 1.002. Whereas endogenous Se can be calculated by multiplying endogenous ⁸²Se by 11.5. Therefore, total Se can be calculated as follows:

\[
\text{Total Se} = \text{exogenous } ⁸²\text{Se} \times 1.002 + \text{endogenous } ⁸²\text{Se} \times 11.5
\]

To show the difference between endogenous and exogenous Se in one figure with the same scale, endogenous and exogenous ⁸²Se were used in this paper.

**Results and discussion**

**Recovery of exogenous ⁸²Se**

We determined the average Se content (three independent samples each) of 13 solid tissues (liver, kidneys, spleen, pancreas, heart, lungs, brain, thymus, submandibular glands, testes, epididymides, seminal vesicles, and muscle), 3 body fluids (plasma, RBCs, and urine), and feces of mice that had been injected with ⁸²Se-enriched SeMet. ⁸²Se recoveries at 24 h after injection were 81% for mice fed with the Se-adequate diet for 4 weeks and 84% for those maintained on a Se-deficient diet. The remained exogenous ⁸²Se could be explained by those in bones, skin, hair, the digestive tract, and the excretion via breath.

**Distribution of endogenous and exogenous ⁸²Se in solid tissues, RBCs, and plasma of mice and excretion in urine and feces under different Se nutritional states**

At the beginning of the tracer experiment (Fig. 1a and 2a), endogenous ⁸²Se levels in the liver, kidneys, and plasma of mice fed with the Se-deficient diet were 3 times lower than those of mice fed with the Se-adequate diet. The decreased Se in the samples from mice fed with the Se-deficient diet likely reflects the insufficient nutritional supply of Se and is consistent with decreased circulating levels of Se. However, reductions in endogenous ⁸²Se in the brain, testes, epididymides, and muscle were relatively small compared with those in other tissues. Because Se plays an important antioxidant role in brain¹⁴ and is involved in sperm production,² we assume that a metabolic pathway works to maintain Se concentrations in these tissues. These uneven distributions of Se into different tissues at different speeds were reported in the case of selenite injection and our data fit well with previous findings.³³,³⁴

Exogenous ⁸²Se was distributed predominantly to liver, kidneys, RBCs, plasma, and muscle during the experimental period (Fig. 1 and 2). In particular, liver was the tissue that accumulated the most exogenous ⁸²Se. This result suggests that liver is important in Se metabolism and that absorbed exogenous ⁸²Se first is delivered to this tissue. After liver, muscle contained the second largest amount of exogenous ⁸²Se. Concentrations of exogenous ⁸²Se in muscle were lower (18.2 ng g⁻¹ for adequate mice and 7.5 ng g⁻¹ for those on Se-deficient diet) than those in other tissues. However when corrected for relative tissue weight (muscle accounted for 45% of body weight), the exogenous ⁸²Se in muscle was a predominant portion of that overall in the body.

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Exogenous $^{82}$Se in liver of mice fed with adequate and deficient diets increased dramatically during the first one hour after SeMet injection (Fig. 1b and 2b). The liver absorbed the injected Se more efficiently than did any other tissue, and we suppose the absorbed Se to be used for the synthesis of selenoproteins or to be excreted from the body. In contrast, exogenous $^{82}$Se was distributed among almost all other tissues at 1 h after injection. We therefore think it likely that another pathway exists whereby the injected $^{82}$SeMet is transferred to other tissues without passing through the liver. Suzuki et al.\textsuperscript{18} reported that isotopically labeled SeMet was detected in rat serum throughout the experiment (from 0.5 to 6 h after oral administration) and concluded that SeMet was taken up by organs in its intact form. This result supports our hypothesis.

Absorption of exogenous $^{82}$Se into the bloodstream of mice fed with the Se-deficient diet was higher than that in mice fed with the adequate diet. In particular, at 6 h after injection, the amount of Se was 1.7 times higher in the plasma of Se-deficient mice than in mice fed with the adequate diet (Fig. 1c and 2c). We presume that the amount of Se in the plasma increased in the Se-deficient mice to supply Se to other tissues.

Previously, at 24 h after injection of $^{82}$Se(IV), 30% and 41% of exogenous $^{82}$Se was retained in the bodies of mice fed with the Se-adequate and Se-deficient diets, respectively.\textsuperscript{22} In contrast, here we noted that 57% and 62% of exogenous $^{82}$Se was retained at 24 h after injection of $^{82}$SeMet in the bodies of mice that received the Se-adequate and Se-deficient diets, respectively. In addition, exogenous $^{82}$Se was delivered to brains of mice injected with $^{82}$SeMet but not $^{82}$Se(IV).\textsuperscript{22} It was reported that selenomethionine is better absorbed than selenite in a high-dose human supplementation trial.\textsuperscript{35} This report supports our results that more Se was retained after the injection of SeMet compared with Se(IV), regardless of the Se nutritional status of the mice.

**Time-dependent changes of endogenous and exogenous $^{82}$Se-species in mice liver and kidneys under different Se nutritional states**

Using size-exclusion chromatography, we noted two major Se peaks (at retention times of 780 and 1180 s) in the hepatic cytosolic fractions of mice at 1 h after $^{82}$SeMet injection (Fig. 3). Comparison with peaks from Se-containing standards revealed the first peak to be cGPx (Fig. S1b, ESI\textsuperscript{†}), but the peak at 1180 s could not be identified through comparison with those for standards of selenosugar (Fig. S1c, ESI\textsuperscript{†}) and SeMet (Fig. S1d, ESI\textsuperscript{†}).

To separate the peaks for selenosugar and SeMet, we used a reverse-phase column. Samples of hepatic cytosol, which was diluted with the mobile phase and centrifuged, obtained at 1 h after $^{82}$SeMet injection yielded two major Se peaks, at retention times of 460 and 900 s (Fig. 4). Subsequent comparison with
standards of SeMet (Fig. S2b, ESI†) and selenosugar (Fig. S2c, ESI†) revealed the first peak as SeMet and the second as selenosugar. The profile of Se-containing molecules in the renal cytosolic fraction was similar to that of liver (data not shown).

The time-dependent changes in endogenous and exogenous 82Se species in liver extracts are shown in Fig. 3 and 4. Exogenous 82Se was associated with cGPx, selenosugar, and SeMet in liver extracts of mice within 1 h after 82Se injection and remained associated with cGPx and SeMet in the liver from 1 to 72 h after injection for both Se nutritional states. Regardless of the diet on which the mice were received, the amount of exogenous 82Se associated with cGPx increased to similar levels within 1 h after injection and was maintained at these levels until 72 h after injection (Fig. 5a and b).

The amount of exogenous 82Se associated with selenosugar in hepatic cytosolic fractions rapidly increased within 1 h after injection and rapidly decreased within 6 h after injection. Selenosugar reportedly is produced less efficiently in the kidneys than in the liver.17 Therefore, selenosugar is produced rapidly in the liver within 1 h after injection of SeMet and then is transported to the kidneys and excreted within 6 h after injection. Exogenous 82Se associated with SeMet peaked in the hepatic cytosolic fractions of adequate mice at 1 h after injection, decreased rapidly between 1 and 6 h after injection, and decreased gradually from 6 to 72 h after injection (Fig. 4).

The amounts of exogenous 82Se associated with cGPx, selenosugar, and SeMet in renal cytosolic fractions were 1/10, 1/6, and 1/3 of those in hepatic cytosol, respectively. However, time-dependent changes of endogenous and exogenous 82Se as cGPx, selenosugar, and SeMet in renal cytosolic fractions showed trends similar to those in hepatic cytosols (Fig. 5c and d).

After Se(iv) injection,24 the amounts of exogenous 82Se associated with cGPx in liver and kidneys peaked at 1 h after injection, decreased between 1 and 6 h, and plateaued from 6 to 72 h after injection. In addition, the amounts of exogenous 82Se associated with cGPx were suppressed under Se-deficient conditions and, even after Se(iv) injection, did not achieve the levels seen under the Se-adequate conditions.24 At 24 h after injection, the amounts of exogenous 82Se associated with cGPx in hepatic cytosolic fractions from mice fed with the Se-adequate and Se-deficient diets were 71 and 79 ng, respectively, after injection of Se(iv)24 compared with 180 and 187 ng, respectively, in mice injected with SeMet.

cGPx activity in Se-deficient cells at 72 h after supplementation with Na2SeO3 was only partially recovered compared with that of Se-adequate cells.26 In contrast, administration of SeMet
at nutritional doses rescued both mRNA and protein expression of cGPx in previously Se-deficient mice to the same levels as in adequate mice.\textsuperscript{37} Corresponding with our results, these previous reports indicate that the amount of exogenous $^{82}$Se present as cGPx in the hepatic cytosolic fraction was greater in mice that received $^{82}$SeMet than $^{82}$Se(IV). That is, the administration of SeMet had a greater effect on the synthesis of cGPx than did Se(IV).

In contrast to the response to intravenous injection of Se compounds, orally administered Se(IV) was incorporated into cGPx and selenosugar more efficiently than was oral SeMet.\textsuperscript{18,19} Similarly, the total Se concentration in tissues after oral administration was greater with Se(IV) than SeMet. These differences suggest that the absorption efficiency of SeMet in the digestive system might be lower than that of Se(IV).

### Time-dependent changes of endogenous and exogenous $^{82}$Se-species in mouse plasma under different Se nutritional states

Heparin-affinity HPLC-ICPMS of plasma from mice revealed two Se peaks (data not shown). The first peak, which was not absorbed by heparin, corresponded to Se-containing compounds other than Sel-P (that is, Se-containing albumin [SeAlb], extracellular GPx [eGPx], SeMet, and selenosugar), whereas the second peak, which was eluted after switching from equilibration buffer to elution buffer, corresponded to Sel-P.

At 1 h after injection, exogenous $^{82}$Se was distributed equally (80 ng) to Sel-P and Se-containing compounds other than Sel-P under both Se nutritional states in our mice (Fig. 6). The candidate Se compounds other than Sel-P in mouse plasma were SeAlb, eGPx, and SeMet. In comparison, the three primary Se-containing proteins in human plasma are Sel-P, SeAlb, and eGPx.\textsuperscript{31,32,38} In light of the presence of SeMet in the hepatic and renal cytosolic fractions of our mice, we suspected that SeMet was transported in its intact form from plasma to the liver and kidneys.

A small peak corresponding to SeMet was detected in rat serum after oral administration of that compound.\textsuperscript{18,19}

At 6 h after injection of our mice with SeMet, exogenous $^{82}$Se was distributed predominantly to Sel-P. In the plasma of mice fed with the adequate diet, exogenous $^{82}$Se of Se compounds other than Sel-P peaked at 1 h after injection and gradually decreased thereafter, whereas exogenous $^{82}$Se as Sel-P peaked at 6 h after injection and gradually decreased thereafter. The increases in exogenous $^{82}$Se as Se-containing compounds other than Sel-P were comparable between mice fed with the Se-deficient and Se-adequate diets, although mice fed with the Se-deficient diet showed about 1.5 times more exogenous $^{82}$Se as Sel-P. Sel-P mRNA levels in rats were higher during Se deficiency relative to Se-adequate conditions.\textsuperscript{39} In another paper, it was reported that Se deficiency down-regulated mRNA expression of cGPx to 15%, while those of Sel-P to only 65%.\textsuperscript{40} These previous findings agree with our results, in which Sel-P was produced more effectively in Se-deficient mice. The important role of Sel-P in Se transport in the body was genetically proven using Sel-P knockout mice.\textsuperscript{41–44} Our results well support the importance of Sel-P in Se transport by exogenous $^{82}$Se incorporation into Sel-P.

We previously surmised that there were two pathways for the transfer of Se after the injection of Se(IV) injection, one involving SeAlb, which was active until 1 h after injection, and the other in which Sel-P was the transport molecule and which was functional between 6 and 72 h after injection.\textsuperscript{22} In addition to these two pathways, another pathway may exist in the case of SeMet injection, in which SeMet is involved from 1 to 72 h after injection. The amount of exogenous $^{82}$Se present as Sel-P increased 1.5 times more in Se-deficient mice than in Se-adequate mice, and this increase was much larger than those seen in Se compounds other than Sel-P.

Trends in the time-dependent changes in exogenous $^{82}$Se as Sel-P in mice were similar after injection of Se(IV) and SeMet. At 6 h after Se(IV) injection, the amount of exogenous $^{82}$Se associated with Sel-P was 93 ng in mice fed with the adequate diet and 160 ng in mice that received the Se-deficient diet.\textsuperscript{22} These previous values are lower than those in mice that were injected with SeMet under adequate and Se-deficient diet states (172 and 243 ng, respectively). These data suggest that SeMet was incorporated into selenoproteins more effectively than was Se(IV).

### Excretion via urine and feces under different Se nutritional states

The time-dependent changes of endogenous and exogenous $^{82}$Se in the urine and feces of mice fed with the Se-adequate and Se-deficient diets are shown in Fig. 7. Speciation analysis of the Se compounds in urine revealed that Se existed only in the chemical form of selenosugar; reverse-phase chromatography failed to detect any SeMet (data not shown). Exogenous $^{82}$Se was excreted predominantly within 24 h after injection regardless of the Se nutritional status. The amount of exogenous $^{82}$Se excreted was greater in Se-adequate than in Se-deficient mice, suggesting that $^{82}$Se was used efficiently in Se-deficient mice. Exogenous $^{82}$Se was excreted via feces within 24 h after...
injection regardless of the Se nutritional status of the mice. Less exogenous $^{82}\text{Se}$ was excreted as feces than urine.

In light of the analytical results from hepatic and renal cytosolic fractions from our SeMet-injected mice, selenosugar rapidly decreased from the liver and kidneys within 6 h after injection regardless of the Se nutritional status. Therefore, most of the exogenous $^{82}\text{Se}$ likely was excreted into both urine and feces as selenosugar within 6 h after SeMet injection. In comparison, excess Se was excreted as selenosugar at 6 h after injection of $\text{Se(IV)}$, with small amounts excreted as Se(IV) and trimethylselenium ions (TMSe$^+$). The time-dependent changes in the excretion of exogenous $^{82}\text{Se}$ via the urine and feces of mice after $\text{Se(IV)}$ injection were similar to those after SeMet injection. These results indicate that Se was excreted predominantly as selenosugar regardless of the chemical form of the Se injected.

**Conclusions**

In the current study, when $^{82}\text{Se}$ was injected intravenously, larger amounts of $^{82}\text{Se}$ were retained in the mice fed with Se-adequate and Se-deficient diet compared to the case of $^{82}\text{Se}$ injection. These results indicate that SeMet injected intravenously is highly bioavailable and is transformed within few hours into selenoproteins, including cGPx and Sel-P. Excess SeMet exhausting the selenoprotein biosynthesis in the liver is excreted as selenosugar.

The Se incorporation into cGPx in Se-deficient mice increased to similar levels to that in Se-adequate mice. Whereas, that into Sel-P in plasma increased 1.5 times more in Se-deficient mice than in Se-adequate mice regardless of the chemical form of the Se injected. These facts imply that Sel-P played an important role in the transport of Se to Se-deficient tissues in mice. In addition to the two pathways for Se transfer identified in mice injected with Se(IV) that is, as SeAlb within 1 h after injection and as SeSel-P from 6 to 72 h after injection—it was suggested that Se was transported as SeMet, which is a readily bioavailable chemical form compared to Se(IV), via a nonspecific Se transport route from 1 to 72 h after injection in the current study.

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**References**


